Polioencephalomalacia in adult sheep grazing pastures with prostrate pigweed

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Abstract — Polioencephalomalacia was diagnosed in 2 animals from different farms. In apparently healthy animals from same farms, fecal thiaminase and a significant reduction in erythrocyte transketolase activity was observed. The presence of thiaminase in *Amaranthus blitoides* could have contributed to the development of polioencephalomalacia in sheep grazing on natural pastures.

Résumé — Polioencéphalomalacie chez le mouton adulte broutant dans des pâturages contenant de l'amarante fausse-blite. Une polioencéphalomalacie a été diagnostiquée chez 2 animaux provenant de fermes différentes. Chez des animaux apparemment en santé des même fermes, de la thiaminase fécale a été retrouvée et la transcétolase érythrocytaire était significativement réduite. La présence de thiaminase dans l'amarante fausse-blite pourrait avoir contribué au développement de la polioencéphalomalacie chez les moutons broutant dans des pâturages naturels.

(Traduit par Docteur André Blouin)

Can Vet J 2005;46:59–61

Amaranthus blitoides (prostrate pigweed) is a naturalized herb listed as a noxious weed and found across parts of the south of Europe, southern Canada, and waste areas through the United States and Mexico (1–3). It is an annual plant, has a mat-like growth, and has branches that spread from 20 to 80 cm. The leaves are oval, from 15 to 20 mm long; they are green or dark red at the base and change to green at the tips. Small, inconspicuous, greenish flowers are found on spikes that rise from the junction of leaf stem and branch. *Amaranthus blitoides* prefers a well-drained fertile soil in a sunny position. It is particularly efficient at high temperatures, in bright sunlight, or under dry conditions (1–3). *Amaranthus* spp. intoxication is rare in the animals; however, there are reports of cattle consuming leaf and portions of the plant and developing nephrotoxicosis (4) and of swine developing perirenal edema and nonspecific degenerative changes in the brain (5).

The Rasa Aragonesa sheep and their crosses are very rustic due to their development in Aragón (northeast Spain), an area classified as continental semiarid Mediterranean. Rainfall is low (300 to 500 mm^3) and, in July and August, temperatures can be as high as 40°C. *Amaranthus blitoides* provides an important food source for sheep in this area. The animals ingest stems, leaves, and seeds. Seeds are very nutritious, rich in starch, but it is rather difficult to crush all of the small seeds in the mouth, thus some seeds pass right through the digestive system without being digested.

Two cases of polioencephalomalacia (PEM) in adult sheep grazing pastures with *A. blitoides* in northwest Spain were reported. The 1st case of PEM appeared in a flock of 700 Rasa Aragonesa cross-bred sheep (meat breed). A 6-year-old sheep developed nervous signs and subsequently died. The clinical signs were characterized by neurological dysfunction that included varying degrees of depression. The affected sheep fell into lateral recumbency with stiffness of the limbs and neck, paddling movement of the limbs, and dyspnea, according to information collected from the sheep owner and the local veterinarian. Significant findings were restricted to the nervous system. The meningeal blood vessels were congested, and the brain showed moderate to severe edema and occasional small foci of hemorrhage. The ruminal contents were normal and contained seeds from *A. blitoides* in association with other plants. On this farm, 2 animals exhibiting neurological signs were treated parenterally with 2 injections of vitamin B_1 (Vitamina B1; Laboratorios Ovejero, Leon, Spain), 10 mg/kg BW, q12h, the first IV and the second SC, which resulted in their clinical recovery within 24 h. On the 2nd farm, a similarly affected 3-year-old sheep was necropsied at the Zaragoza Veterinary School. Necropsy revealed pulmonary edema and congestion, as well as small epicardial hemorrhages. The other major organs appeared normal and the ruminal contents showed an abundance of seeds from *A. blitoides.* The brain showed moderate to severe congestion, edema, and numerous yellowish foci of varying sizes scattered on the surface of the cerebral hemispheres; affected foci were soft and friable in comparison with surrounding unaffected foci.

The brains and sections of the cerebral cortex from both sheep were examined under an ultraviolet light lamp, with an emission wavelength peak at 365 nm, which revealed segmental and multifocal areas of autofluorescence.

Samples of ruminal contents and feces from the animals autopsied were quickly frozen at -20°C and the cerebrums were collected in 10% neutral buffered formalin for histopathological studies. From pieces of the cerebrum, 5-μm sections were cut and stained with hematoxylin. Microscopically, both samples showed very similar changes: cortical neuronal degeneration and liquefaction necrosis of the affected cerebral cortex, with eventual removal of necrotic cortical tissue by phagocytes (gitter cells). Neurons in the cerebral cortex showed

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chromatolysis and pericellular edema with eosinophilic cytoplasm and vacuolization. Endothelial and perithelial cells of existing capillaries showed hypertrophy and, within affected and adjacent tissue, a few lymphocytes, monocytes, and plasma cells were present.

The signs of nervous disorder, recumbency, and the pathological features, including the swelling and necrosis of the grey matter of the cerebrum, were comparable with those reported for sheep with PEM (6,7).

Information on the affected animals, health program, and husbandry practices were collected, with particular focus on the pastures on which the affected animals had been grazing before the disease developed. The 2 flocks were treated with oral ivermectin (Oramec; Merial, Lyon, France), 0.25 mL/kg, and vaccinated against enterotoxemia (Miloxan; Merial Laboratorios, Barcelona, Spain). In the 1st flock, the sheep had been grazing new pastures and dry land cereal stupple fields for 3 wk. The following species made a significant contribution to the pasture mix: *Hordeum* sp. (fresh barley), *A. blitoides* (prostrate pigweed), *Erodium cicutarium* (filaree), *Chenopodium album* (lamb's quarters), *Senecio vulgaris* (groundsel), and others. On the 2nd farm, the sheep had been grazing for 5 wk on Mediterranean type xerophytic pasture, plains, and plateaus with *Thymus vulgaris* (thyme), *Rosmarinus officinalis* (rosemary), *Lygeum spartum* (esparto grass), and *Ulex europeaus* (furze), and at the same time, on dry land barley stubble fields with the dominant presence of *A. blitoides* and *Salsola kali* (russian thistle).

Pasture samples (approximately 1 kg of each plant species) from the area grazed by the affected animals were collected for thiaminase analysis. *Equisetum arvense* (horsetail), a plant with known thiaminase content, was included for comparison purposes. Water samples were collected in plastic vials for sulphate analysis by a commercial colorimetric method (Aquanal-plus Sulfat; Riedel-de-Häen, Seelze, Germany).

Blood samples and feces were obtained from 10, randomly selected, apparently healthy sheep from each flock. The blood sample from each animal was distributed into 3 tubes containing fluoride-oxalate for lactate analysis (Lactate 735; Sigma Diagnostics, St. Louis, USA), perchloric acid for pyruvate analysis (Pyruvate, kit number 726-UV; Sigma Diagnostics), and heparin for erythrocyte transketolase activity (ETKA), respectively. The ETKA was determined in the absence of thiamine pyrophosphate (TPP) and in the presence of added TPP (effect TPP) by a fluorimetric method (8). Samples were also taken from 1 herd that had no cases of PEM on pasture.

Total thiaminase activity in the postmortem samples of ruminal fluid, feces, and plants was determined in duplicate by using the radioactive method of Edwin and Jackman (9), modified by Edwin (10), in which thiazole-[2-^{14}C]-thiamin hydrochloride (Amersham International, Little Chalfont, United Kingdom) is used as a substrate.

Differences between affected flocks and control groups were calculated by a Kolmogorov-Smirnov nonparametric analysis. The median and interquartiles ratios were calculated.

Polioencephalomalacia has been induced in ruminants by feeding a thiamine-deficient diet, through salt toxicosis (water deprivation), or due to a high sulphur intake (7). Despite multiple causes, PEM has traditionally been believed to result from thiamine deficiency caused by a lack of de novo rumen microbial synthesis; ingestion of thiamine antagonists, such as amprolium; or thiaminases (ruminal or vegetal origin) that could destroy thiamine (7).

There are some hypotheses that have suggested that PEM may be caused by the administration of amprolium, an antagonist of thiamine, or by sulphur or lead intoxication. In the present study, the flocks had not been dosed with amprolium, the sulphate content in the samples of drinking water was < 50 ppm, and there was no evidence suggesting a lead intoxication. There were no potential sources of lead in the environment, and the necropsied animals showed no pathological changes in their livers, kidneys, or small and large intestines. The response to treatment with thiamine in 2 animals may also be an aid in a tentative diagnosis.

In these cases of PEM, thiamine deficiency as the cause of the disease is supported by the recovery of spontaneously affected animals following thiamine therapy. The occurrence of thiamine deficiency is most probably related to the presence of thiaminase. In the 2 necropsied animals, rumenal and fecal thiaminase activities were 14.24 and 11.29 $\times$ 10^{-4} μmol thiazol/min (1st case) and 13.08 and 6.66 $\times$ 10^{-4} μmol thiazol/min (2nd case), respectively. Thiaminase was also found in 100% of the feces samples from clinically normal sheep in the same herds in which the sheep had succumbed to PEM.

The presence of thiaminase has been associated with feedstuffs containing thiaminase, with feeding carbohydrate rations, or with abrupt changes in diet or grain engorgement (7). In the present study, there was no history of feeding concentrates and the cases of PEM occurred after a prolonged period of grazing on grass pastures.

Thiaminases are enzymes found in a few plants. A plant often associated with cerebrocortical necrosis (CCN) in Australia and the USA is bracken fern (*Pteridium aquilinum*) (11). In addition to bracken fern and horsetail (*Equisetum ramosissimun*), kiesieblaar (*Malva parviflora*) may also contain thiaminases (12). Thiaminase activity was found only in *A. blitoides* collected from both areas grazed by the animals: sample 1 (leaves 17.02 μmol thiazol formed $\times$ 10^{-4} and seed 13.47 μmol thiazol formed $\times$ 10^{-4}), and sample 2 (leaves 14.67 μmol thiazol formed $\times$ 10^{-4} and seed 8.06 μmol thiazol formed $\times$ 10^{-4}). The thiaminase activity was similar to that found in *Equisetum arvense* (12.50 μmol thiazol formed $\times$ 10^{-4}). Fresh barley (*Hordeum* sp), *Erodium cicutarium, Chenopodium album, Senecio vulgaris,* and *Salsola kali* all showed negative thiaminase activity.

The thiamine concentration was not measured in the present study, but in a previous study in lambs, the transketolase test and the TPP effect appeared to offer the best approach to the assessment of thiamine status in vitro for early diagnosis of a subclinical thiamine deficiency (6). The decrease in the ETKA ($P < 0.01$) was accompanied by a corresponding rise in the TPP effect ($P < 0.01$) in apparently normal animals from the farm 1 (Table 1).

Table 1. Median and interquartiles ratios (IQR) of blood pyruvate, lactate, erythrocyte transketolase activity (ETKA), thiaminepyrophosphate (TPP) effect, and fecal total thiaminase activity in apparently healthy sheep from problematic and control farms

	Flock 1 $n = 10$	Flock 2 $n = 10$	Control $n = 10$
Pyruvate (μmol/L)	12.32, IQR = 5.11	13.17, IQR = 2.50	11.47, IQR = 1.82
Lactate (mmol/L)	2.83, IQR = 1.14	3.22, IQR = 0.67	2.32, IQR = 1.00
ETKA (mU/g Hb)	288, IQR = 73[b]	467, IQR = 60[a]	618, IQR = 166
TPP effect (%)	76.5, IQR = 17.0[b]	33.5, IQR = 7.0	31.0, IQR = 10.0
Fecal thiaminase (μmol thiazol formed $\times 10^{-4}$)	14.9, IQR = 3.26	4.77, IQR = 3.25	Negative

[a,b]Significantly different from the control group at [a]($P < 0.05$), [b]($P < 0.01$)

The disease occurs only sporadically, and in this region, it is well known that sheep may be able to ingest considerable amounts of *A. blitoides,* leaf and stem material, for long periods without ill effect. The necropsied animals showed abundance of seeds from *A. blitoides* in the rumen. More research is needed to elucidate the level at which the thiaminase affects sheep and the concentrations of thiaminase in *A. blitoides.* These could change depending on growth characteristics of plants or animal conditions.

In conclusion, thiamine deficiency was the most probable cause of PEM observed in these sheep and the presence of thiaminase in *A. blitoides* could contribute to the development of PEM in sheep grazing on natural pastures. CVJ

Acknowledgments

The authors thanks Ms. C. Sanz-Marqués and P. Noone (Instituto de Idiomas — Universidad de Zaragoza) for linguistic advice.

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